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AUTOCLAVABLE DISPENSING DEVICE

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AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY
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FOR THE COMMANDER

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This device described has proved to be of great value in our laboratory. The savings in time expended not only on addition of the glucose but also in the re-doing of contaminated bottles has been significant.

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PREFACE

This study was conducted in the Toxic Hazards Division, Environmental Quality Branch, Air Force Aerospace Medical Research Laboratory. This research was performed in support of Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations", Task 04, Workunit 19, from May 1979 to September 1979.

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INTRODUCTION

Five BiFlo® model C-30 chemostats for continuous growth of bacteria have been in use since the early 1970s at the Environmental Quality Branch of Air Force Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. These chemostats are part of an overall effort by the Branch to evaluate and better understand the environmental impact of various chemicals including hydrazines, jet fuels, and lubricants that are currently in use by the United States Air Force. Presently the chemostats are used as a screening procedure in which dose-effect levels are ascertained from turbidity readings.

These experiments, usually two weeks in duration, require 13-liter glass jugs to store the nutrient for the bacteria without replenishment. In using a medium volume this large, other problems were encountered which had to be resolved. One of these problems was dispensing filter-sterilized glucose stock solution, which served as the carbon source for the cultivation of the bacteria used in the testing procedure.

Since turbidity was a parameter to be measured in the study, the medium should be as clear as possible prior to beginning the experiment. This would enable the detection of subtle changes which might occur during the course of the experiment. Since all components of the medium (London, 1977) except glucose could be autoclaved together without an increase in turbidity, we decided that the glucose should be added after autoclaving. In order to accomplish this, a filter-sterilized glucose stock solution was prepared. This could then be added to the medium aseptically after sterilization of the other components.

The glucose was added to the nutrient jugs with disposable sterile syringes and needles. This procedure was time consuming, required a large supply of needles and syringes, and very often resulted in contamination of one or more of the nutrient carboys. To alleviate most of these problems, we designed and fabricated an autoclavable dispensing device using common and inexpensive laboratory supplies. This paper describes the design and assembly of this device.

MATERIALS AND METHODS

Equipment: The equipment needed to assemble the device shown in Figure 1 is listed below.

1. Erlenmeyer flask, 1000 mℓ
2. Erlenmeyer flask, 125 mℓ

3. One #5 stopper (rubber)

4. Two teflon holding straps

5. One stainless steel needle, 18-20 gauge

6. Two stainless steel 3-way valves

7. One stainless steel 2-way valve

8. One length of silicone tubing, 6 cm (0.5 cm I.D.)

9. One length of transmission tubing, 0.5 m (0.5 cm I.D.)

10. Two disposable (sterile) syringes. These may be varied according to the needs of the laboratory

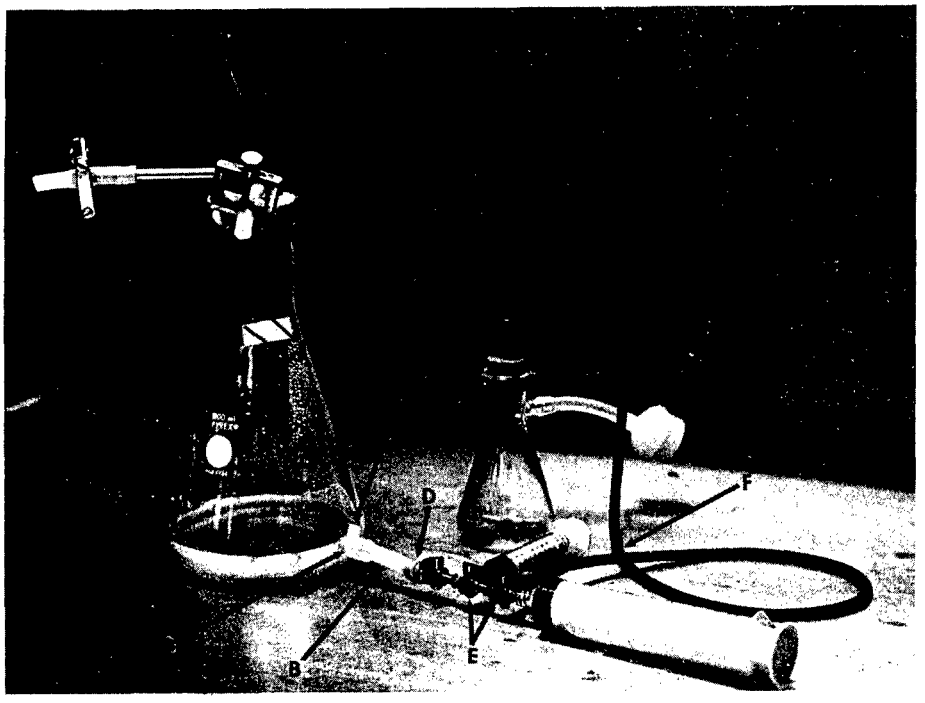

Figure 1. Autoclaving Dispensing Device.

Design and Assembly: 1) A hose connector (A) is attached to the base of the 1,000 mℓ Erlenmeyer flask; 2) the piece of silicone tubing (B) is attached to the hose connector and secured in place with one of the teflon (C) holding straps; 3) the 2-way valve is then connected to the silicone tubing and secured with the second teflon (D) holding strap; 4) next, the two 3-way valves (E) are connected in series with the 2-way valve (note that these three valves are welded in place in order to prevent leakage and to help maintain the sterile integrity of the system); 5) when the valves are thus connected, the transmission tubing (F) is attached to the side connection of the second 3-way valve; 6) the disposable syringes (G) are connected at the side of the first 3-way and at the end of the second 3-way valve; 7) the other end of the transmission tubing is fitted with the needle (H); and 8) attached through the stopper (I) into the 125 mℓ flask (J). This 125 mℓ flask acts as a sterile receptacle for priming of the syringes and the transmission tubing prior to use (refer to Figure 1 for the complete assembled device).

Autoclaving the Device: Before the device is used it must be sterile. This sterilization is accomplished by autoclaving the device at 15 psi and 121°C for a period of at least 15 minutes. The device can be autoclaved as a unit, with the exception of the syringes. These must be removed and their attachment points covered with cotton stuffed silicone tubing of the same dimensions used previously. After autoclaving, these two pieces of tubing are removed using sterile technique, and replaced with the appropriate syringes.

Filling the Device Prior to Use: After the device is autoclaved and the syringes are attached at their proper places, the device is filled with the filter-sterilized glucose. This is accomplished by first withdrawing the needle from the 125 mℓ flask and inserting it into the flask containing the glucose stock solution (Caution: use sterile technique at this point or the device as well as the stock solution may be contaminated). A vacuum line is then attached to the filter at the top of the receiving flask and a vacuum applied. All valve stopcocks are turned so that they allow the glucose from the stock flask to flow into the receiving flask. When the desired amount is transferred to the receiving flask, the vacuum is turned off and allowed to dissipate in the flask before the vacuum line is disconnected. The device is now ready for use.

Addition of Glucose to the Medium: At this stage, addition of the glucose is accomplished in two steps. First, the valves are opened so that the glucose may be drawn into the two syringes. Secondly, these valves are adjusted to close off the flask. Glucose from one syringe and the contents of the second syringe are injected into the medium. The order in which the glucose is dispensed is completely arbitrary. This process may be repeated as many times as required to complete the addition of the desired volume of stock glucose solution. When completed, the device is rinsed well with distilled water, the syringes removed and destroyed, the cotton-stuffed tubing replaced, and the unit autoclaved for re-use.

SUMMARY

Use of this device in our laboratory has been exclusively for the addition of sterile glucose to the nutrient media reservoirs for the chemostat continuous culture system. The device has proved particularly useful because the glucose stock solution used in our laboratory is filter-sterilized in very concentrated form prior to use. We prefer this procedure due to the caramelization of the glucose when autoclaved.

Glucose must be added to the mineral salts media in an aseptic manner and by using the device described, contamination has been virtually eliminated. A tremendous saving of time and materials has also been realized. When the device was first tested in our laboratory, the addition of approximately 1,200 mℓ of glucose to 13 nutrient reservoirs took about 15 minutes and required only two syringes for completion. Previous methods for this same addition would have taken from two to three hours and a minimum of ten syringes and needles and would very often result in one or more contaminated bottles.

Use of this device is not limited to the specific application outlined; it can easily be used in any application requiring sterile delivery of non-autoclavable liquids.

REFERENCES

London, S.A., 1977. A System for Chronic Toxicity Studies with Microorganisms. Proc. 8th Ann. Conf. Environ. Toxicol. AMRL-TR-77-97, Paper Number 19, Pages 261 - 280. Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. (AD A-051334)

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